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Nereocystis

by

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Nereocystis

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INTRODUCTION

For the last few years botanists who have been keenly interested in the algae have had their attention largely centered on the Phaeophyceae. Part of this group, the Laminariales, altho having some of the characteristics of the higher plants, has long been looked upon as having a rather primitive life history.

Drew[1] in 1910 reported fusion of motile gametes of Laminaria, figured resulting plants and described their growth. Williams[2] in 1913 repeated the experiments of Drew. His observations did not quite coincide with those of Drew in that he did not find the fusion of the motile spores, produced by the large Laminaria generation. He concluded that the colorless fusing bodies that Drew described were not spores of the plant in question. He calls these bodies monads. Williams also described the young plant resulting from the Laminaria spores together with a subsequent development from an extruded cell of a more complex body, which he calls a germling.

It remained for Sauvageau[3] in 1916 to interpret these facts thru his cultural study of another genus, Saccorhiza bulbosa. He reported a differentiation in the little sporelings and called them male and female gametophytes. The extruded cell which Williams had observed in Laminaria was present here and gave rise to a plant similar to that described by Williams. Sauvageau rightly conceived this extruded cell to be the egg, and the small plant resulting from it to be the sporophyte. He decided that the other small cells (often empty) developing on the smaller, less complex plant, were antheridia. He followed this study in 1916 with like observations on Laminaria

1 Drew, G. H. The reproduction and early development of Laminaria digitata and Laminaria saccharina. Ann. Bot. 24:177-190. 1910.

2 Williams, J. Lloyd. The zoospores of Laminariaceae and their germination. Rept. 82. Meeting of British Assn. Adv. Sci., Dundee. 1912.

3 Sauvageau, C. Sur la sexualité heterogamique d'une Laminaire (Saccorhiza bulbosa). Compt. Rend. Acad. Sci. Paris 161:746-799. 1915.

saccharina, L. flexicaulis[4] and Alaria esculenta.[5] Kylin[6] in the same year confirmed Sauvageau's observations in his study of Laminaria digitata (L. flexicaulis), and in 1918[7] summed up the facts concerning the alternation of generations in the Phaeophyceae, giving a comprehensive bibliography.

In a preliminary paper in 1921 Williams[8] completed the chain of evidence for alternation of generations thru the discovery of the free sperms and the fertilization of the egg in Laminaria and Chorda. Thus the conception of this great group was changed and alternation of generations became a firmly established fact.

Myers[9] in 1925 published the life history of Postelsia palmaeformis and of Laminaria sinclairii, and in 1928[10] that of Egregia menzesii. In the latter paper she established alternation of generation by chromosome count. Delf and Levyn[11] in 1926 published a report on alternation of generations in Macrocystis pyrifera, and Angst[12] in 1927 described it in the development of Costaria costata. The life histories of the closely related genera are still only conjectural.

The purpose of this paper is to gather the existing literature on Nereocystis luetkeana (Mertens) Postels & Ruprecht, and to set forth its life history as determined by cultural methods.

History

Nereocystis luetkeana, the only species of the genus, was first described by Mertens (1820), under the name Fucus lütkeana; however, Setchell (1908) refers to a rare edition of Maurelle's "Journal of a voyage in 1775 to explore the coast of America northward from California." This author tells of a floating sea weed, the description of which answers to that of Nereocystis. As the latitude and longi-

4 Sauvageau, C. Sur les gametophytes de deux Laminaires (L. flexicaulis et L. saccharina). Compt. Rend. Acad. Sci. Paris 162:601-604. 1916.

5 Sauvageau, C. Sur la sexualité heterogamique d'une Laminaire (Alaria esculenta). Compt. Rend. Acad. Sci. Paris 162:840-842. 1916.

6 Kylin, H. Ueber den Generationswechsel bei Laminaria digitata. Svensk Bot. Tids. 10:551-561. 1916.

7 Kylin, H. Studien ueber die Entwicklungsgeschichte die Phaeophyceen. Svensk. Bot. Tids. 12:1-64. 1918.

8 Williams, J. L. The gametophyte and fertilization of Laminaria and Corda. (Preliminary account). Ann. Bot. 35:603-608. 1921.

9 Myers, M. E. Contributions toward a knowledge of the life histories of the Melanophyceae. (A preliminary report). Univ. Calif. Pub. Bot. 13:109-124. 1925.

10 Myers, M. E. The life history of the brown alga, Egregia menziesii. Univ. Calif. Pub. Bot. 14:225-246. 1928.

11 Delf, E. M. and Levyn, M. Reproduction in Macrocystis pyrifera. Ag. Ann. Bot. 40:503-506. 1926.

12 Angst, L. Gametophytes of Costaria costata. Pub. Puget Sound Biol. Sta. 5:293-308. 1927.

tude was that of the Alaskan coast, it is very probable that he was describing the Nereocystis beds.

Postels and Ruprecht (1840) first described the plant under the name Nereocystis lütkeana. Harvey (1852) refers to Nereocystis, giving Mertens' description in full, and adds that the plant seems to be confined to "Russian America." Areschoug (1876) contains the first description of the fruiting structures and the young plants. Macmillan (1899) added to this knowledge of the young plants when he examined hundreds of Nereocystis plants from a collection made by Tilden at low tide at a depth 60 cm. He states that the series "includes undoubted specimens from ½ mm in length to 80 feet" (25 meters).

From this time Nereocystis has been the subject of many papers. Its abundance and possible utilization as a source of potassium salts and iodine has led to investigations by individuals and governments to determine its value and the extent of its beds. In the bibliography given at the conclusion the writer has endeavored to include all papers resulting from such investigations as well as those of purely scientific purport.

Cultural work on the problem was begun by the writer in 1925 and repeated in 1925-1926 with some success, but the slow growth of the young gametophytes, the absence of good working conditions, and the distance from the Nereocystis beds, made the problem a difficult one. In 1927 cultures were set up at the Puget Sound Biological Station under more favorable conditions. The results of the combined data from all of these experiments form the basis of this paper.

General Description

Nereocystis grows only in the Pacific Ocean off the coast of North America. Jordan (1896-1897) in speaking of the range of the plant says, "It certainly extends as far north as Unalaska and may very likely range as far up as the Pribilof group." Setchell (1912) gives as its range, Point conception, California to the Shumagin Islands, Alaska. The beds vary in size and somewhat in location from year to year. The bed at Lincoln Beach, Seattle, from which most of the material was collected during the winters of 1924-1926, has been described by Rigg (1915a). This bed is not so extensive as some of those in the neighborhood of the San Juan Islands, but at all times supplied reproductive material for the setting up of the cultures. In the period from February to the last of May reproductive

material was not abundant, since most of the plants disappeared during the winter months.

The adult sporophyte consists of a complex holdfast made up of branching, intertwined hapteres and a long stipe topped by a hollow bulb from which the numerous fronds arise dichotomously. The fronds vary in length and number according to age.

When the fronds are mature they contain areas which have a thicker, heavier, more leathery apearance than that of the rest of the plant. As these patches near maturity, they become quite noticeable, varying in size from 2.5-10 cm in width and often reaching a length of 45 cm. These are the fruiting areas. They may be found in a series on the same frond, from those which are just forming and scarcely discernable to those mature, dark, olivaceous ones which are ready to shed. The older ones are nearer the tip of the frond; when the zoospores are mature the patch separates from the frond, leaving a hole. The frond is thus weakened by perforations, and becomes ragged and frayed by action of wind, waves and tide.

By the first of April small plants with fronds a meter long may be found in the edge of the water just beyond low tide. Plants of all sizes from a centimeter up may be collected at this time.

As the plants near the shore are mixed with Laminaria and other large browns, one cannot be sure of their identity, at least by macroscopic observation, until they have reached a length of about 10 cm. At this time the stipe has become one-third the length of the whole plant; the little lines along which the frond splits some time later have begun to be evident, and a slight bulge in the stipe indicates that the pneumatocyst is forming.

In artificial cultures sporophytes reached a length of 1.5 cm in 10 months from the time when the first sporophyte appeared. Their growth was probably retarded by the confines of the dish, their too great abundance in the cultures, together with other conditions not met with in nature. Of course, growth cannot be so rapid at this stage, as in later stages when the cells number many times those of the small plant.

The Nereocystis plant, when full grown, varies in size according to the depth from which it comes and according to other determining factors of which Setchell (1912) and Rigg (1913) conclude salinity to be one. Frye, Rigg and Crandall (1915) found them reaching a total length of 38.4 meters, and growing at a depth of 17 meters.

The length of the life of Nereocystis is still uncertain but the slow growth of the gametophyte, its age at maturity, and the subsequent slow growth of the sporophyte in cultures has led the writer to agree with Frye (1906), who suggests that Nereocystis may not reach the surface the first year.

Methods of Culture

The culture solutions were all made on the same general plan. In each dish was placed .5-1 liter of sea water to which had been added sodium nitrate in the ratio of one gram per liter and a crystal of potassium phosphate about half the size of a grain of wheat. The sea water was either filtered or sterilized; if sterilized it was placed in an autoclave or pressure cooker, brought to 10 pounds pressure and left for 20 minutes; if filtered, it was run thru a fine filter several successive times in order to remove everything that might interfere with the clearness of the cultures.

Cultures set up with sterile sea water were freer of bacteria, diatoms, plant and animal life, and this method was therefore used in all the later experiments. Slight variations in the amount of sodium nitrate showed that in .1% to .15% solutions the plant growth was fairly constant. Where less than .1% was used the cells of the gametophyte were spherical, quite uniform in size, and the filaments did not grow rapidly or branch. Slight variations occurred in some of the other solutions, but because so many factors are encountered in general cultures, no conclusions could be drawn.

As the .1% sodium nitrate solution used by Kylin on Laminaria seemed to be favorable for the growth of the small Nerecocystis gametophytes, this amount was used in all the later cultures. Acme section jars were chosen for the experiments, as these could be placed one above the other. However, the method of placing them one above the other was early given up when it was found that the cultures did not thrive so well as when surrounded by a greater amount of air. In order to increase the amount of air after sterilization, the water was poured back and forth several times from a height of 20-30 cm. As many slides as could be accommodated were then placed on the bottom and around the sides of the dishes.

When the solutions were in readiness, trips were made to the Nereocystis beds. During the winter of 1924-1925 and that of 1925-1926, all material was collected from Lincoln Beach which lies about ten miles from the University of Washington campus. During the

summer of 1927 at the Puget Sound Biological Station, the reproductive portions of the fronds were obtained from a small bed off Point Caution, about a quarter of a mile from the Station float. Since plants nearer the shore were covered with dense growths of Ectocarpus, the material was taken, for the most part, from plants that grew in deep water.

The reproductive parts were collected just as they were dropping from the blades and brought to the laboratory in plenty of fresh sea water. Pieces .5-1 cm square were cut, washed several times in filtered or sterile water and placed in the culture solutions previously described. Only three or four pieces of this size were placed in each dish. Small pieces and large amounts of solution were used, that the danger of contamination by other filamentous brown algae might be minimized. So thoroly was the washing done that cultures practically pure, except for protozoans and bacteria, were secured.

In 1924-1925 the cultures were then placed out-of-doors and covered in such a manner that they were protected from dust and at the same time were constantly supplied with fresh air. To accomplish this the dishes were placed on an open grating and covered with a large bell jar. Cultures set up in this way in September usually died because at that time the temperature varied too greatly. For this reason cultures were set up at intervals of a week or two weeks until the first of December.

In 1927 at the Biological Station, the temperature was more easily regulated. The dishes were placed in a shallow cement tray and surrounded with sea water conveyed to the laboratory from a storage tank by a pipe that runs for some distance thru the cold water of Puget Sound. A faucet above the tray supplies the inflow; the outlet allows for the control of the depth of water in the tray. The water was maintained at a fairly constant temperature. At no time did it register higher than 18°C. with an average of about 16°C. The pieces of reproductive material in the cultures were removed after 12 hours. By this time enough zoospores had liberated themselves to make a thin brown film on the top of the water in the dishes. A drop of water on a slide showed myriads of tiny brown moving forms.

Permanent slides were then made by placing a drop of the solution on a slide, putting the slide over osmic acid in a closed container, leaving it for five minutes and then drying the slide slowly in the air. The carbol-fuchsin method was used in the staining. This

gave the chromatophores, together with the cilia of the zoospores, a bright red color, and stained but slightly the wall and protoplasmic contents.

The zoospores as seen from a stained prepared slide were ovoid with a slight depression on the side nearer the smaller end, from which depressions arose two cilia of unequal length, the shorter one being about the length of the spore itself, the other twice the length (fig. 1). The body of the zoospore measured 3.8-4.2 micra in length, by 1.5-3.1 micra in width. It contained one large, rather irregular chromatophore parietally located near the smaller end of the spore.

The zoospores in fresh cultures swam about quite actively for a short time, then rounded up and provided themselves with a wall. They settled on the slides, on the bottom or sides of the dishes, or floated in great numbers on the surface of the water. They adhered closely in groups when in this floating condition.

Gametophyte

In 24 hours after the reproductive material was placed in the dishes many of the small spores began to germinate by putting out a short projection. The tube which formed from this was much smaller in diameter than the spore itself. By the second day most of the contents of the cell had pushed into the filament thus formed, and the end had swelled, giving the whole the appearance of a tiny dumb-bell, similar to that shown in drawings for other genera of the Laminariaceae by Sauvageau and others (figs. 4, 5, 6). Soon, with the exception of a small amount of protoplasm, the contents of the spore became the contents of the swollen end. This now formed a wall and the first cell of the young gametophyte was completed (fig. 7). At this stage the cell had two or more chromatophores.

For several days the cell increased in diameter and in number of chromatophores. It then started to grow by a process similar to that seen in the spore except that the contents of the cell became dense and remained so. The filament instead of rounding up at the end remained the same diameter and the first cell divided it into two almost equal parts (fig. 14); at about the same time a wall appeared separating the basal portion from the filament of two cells, thus forming a three-celled gametophyte (figs. 20, 21, 22). The division of the cells in the filament continued. Up to the time that the filament was 6-8-celled the cell division caused increase in length only,

and this growth in length was entirely in cells other than the basal. This cell, however, then rounded up, increased in size until its diameter was 1.5 times that of the filament. It sometimes divided and formed a wall diagonal in direction to those in the filament. The first division of the basal cell was often unequal. At right angles to the diagonal wall might appear another wall in the larger cell. Subsequent cell division created a mass of cells from each of which a branch might be sent out, giving the whole a rosetted appearance (fig. 48). At other times the basal cell just remained a noticeably larger cell and the cells in the filaments sent out branches. Often all the cells in the filaments formed branches. These branches were at times all on one side, at other times some on one side and some on the other; again there was only an occasional branch in the whole gametophyte.

In some cases all of the cells in a gametophyte died but one. That one might increase in size and number of chromatophores and then give rise to a new plant dense with protoplasm and brown in color. The cause of the death of the filament or of the rejuvenation of the plant by the remaining cell was not apparent, altho this was a common occurrence in cultures that were otherwise seemingly healthy. At the time the cultures were 3-4 weeks old, the sides and bottoms of the dishes were brown with the growth. The surface had a thick, brown film, which, when viewed under the microscope, was seen to be composed of sturdy plant filaments. They seemed to thrive equally well in all parts of the dishes.

In cultures 3 weeks old there was still belated germination of the asexual spores (fig. 18), suggesting that perhaps these heavier structures had resulted from spores that had a longer maturation period. From time to time cultures that seemed to be laboring under adverse conditions degenerated into filaments of rounded cells with slightly heavier walls. In these cultures but little branching was seen.

The slides in the bottoms of the dishes were removed from time to time and made into permanent mounts. The small plants adhered so firmly to the slides that they could be killed and run thru the different staining processes without being loosened. At the end of 4 weeks the slides had such a growth of young plants that together with the debris that collected from different sources they were not worth much as permanent slides. From this time forth, if the material was desired for permanent mounts, it had to be treated in another manner.

Very little differentiation of the plants was noted before they were 9 or 10 weeks old. Then some of the plants became noticeably stouter in cell structure, more complex in their branching, denser in protoplasm and chromatophores and correspondingly darker brown in color.

Cultures set up by Myers in 1924, but abandoned at the end of the summer, were still growing in 1925. They were again supplied with sterile water and nutrient salts, and left until the next summer. They were still living in the gametophyte stage in the early part of the summer of 1928. Each summer they were taken care of in the same way. No attention was paid to the temperature, and the air space left in the dishes was small. These cultures showed two differentiated structures. One consisted of small round cells, as was the case also in some of the writer's cultures. The other was an unbranched, very much smaller filament with slightly longer cells, which was somewhat similar to the male gametophyte in some of the cultures set up by the writer. There were no sporophyte structures in the Myers cultures and care during the summer of 1927 and thruout the year until 1928 did not produce any, at least not in the 9 weeks that they were under observation each summer. At the end of the summer in 1928, after being carefully nourished and kept at the constant temperature, as were my own cultures, the cells had taken on the dark brown coloration and were much the same in appearence as the gametophytes in cultures that were producing fruiting bodies.

Cultures set up on July 1st, 1927 at the Puget Sound Biological Station had not produced oogonia or antheridia when the time came to leave the Station, so they were left in the care of Martin Johnson, the curator, to collect and send samples to the writer at the University of Washington. The samples of the 11th of September contained the fruiting structures, exhibiting the quite apparent differentiation of the two gametophytes.

Female Gametophyte

This differentiation showed in the size and to some extent in the color of the filaments. Some of them had taken on a sturdiness of form not so apparent in the earlier structures. These gametophytes had increased in diameter, and were a richer brown in color due to the increased numbers of chromatophores. The walls of the cells had a somewhat heavier appearance. The apical cells of many of the branches were several shades darker than the rest of the filament.

Their length was much augmented and the apex of the cell was decidedly swollen. This made the oogonial structures, as these later proved to be, strikingly noticeable. From the ends of several empty cells in this culture 1- or 2-celled sporophytes could be seen dotting the cultures here and there.

In observations on previous cultures and on these cultures in a live state in the summer of 1928, the writer often saw the oogonial cell empty its contents. The cell when mature had its contents crowded into the apex. Behind and around this contents the cell was filled with a dense colorless substance. The cell broke at the tip and the mass of material contained in it moved out into the surrounding water. As early as 1916 both Sauvageau and Kylin observed the same phenomenon in Laminaria. This extruded cell seemed to have a membrane surrounding it, for after flowing out thru the small opening, it immediately rounded, adhering closely to the empty oogonium. It then formed a cell wall about itself and began to elongate. The fertilization has not been observed nor has cytological work been done on the plant.

In older cultures oogonia occurred not only in the tip cells of the branches; any cell of the filament might elongate at right angles to the main axis and become an oogonium. As the cultures grew older, all the cells of the female gametophyte took on the dense brown color, and the oogonium was less distinctly noticeable than in the younger cultures. By the time the oogonia were formed the gametophyte was an elongated and much branched structure, or had become a decidedly resetted structure with many branches (fig. 48), the tips of which produced the oogonia. One plant could give rise to many sporophytes and as the plant could reproduce vegetatively from a singly cell, limitations on sporophyte production did not seem to exist.

When the cultures had been newly supplied with much nutritive solution, or continuously supplied with fresh sea water from the tap, an interesting development occurred in older cultures of the gametophyte. This was the formation within the filaments of giant cells 140 by 45 micra. These giant cells in some cases just died without much change; sometimes they sent out 1 or 2 thrifty branches from their sides; and at other times they divided into many cells, forming plate-like structures the branching of which produced rosettes of filaments from common centers, similar to those made by the division of the basal cells of the gametophytes.

Male Gametophyte

Very intimately associated with the above sturdy, coarse filaments were plants much more delicate in appearance. They were paler in color, contained the same type of laterally placed chromatophores, had the same kind of branching filaments and became very extensive before they began to be interesting in a reproductive way.

At first the antheridia were formed at the tips of the branches and consisted of finger-like projections rounding out from much divided and plate-like or spherical structures formed from many divisions of the apical cells (figs. 40, 41). The contents of the outermost cells then rounded up and produced single sperms. This seemed to be the condition as observed in most of the Laminariaceae.

The whole much-branched antheridial portions of the male gametophytes gradually emptied as the cells successively produced sperms and the latter escaped. The escape was not observed; therefore, whether the forms which emerged were free-swimming is still to be determined. The evidences that there were sperms were the presence in the cultures of the antheridial structures, the single small bodies in the cells, and then the empty cells after the sperms had abandoned them. Observations by writers on other genera also led to this conclusion.

Sporophyte

The sporophytes began to appear when the cultures were between 10 and 11 weeks old. After the egg left the oogonium it rounded up. A wall formed about it and it began to grow. The first indication of growth was the elongation of the cell to almost twice its former length (fig. 42d), after which a wall appeared at right angles to its main axis, dividing it into two either equal or unequal parts. If unequal, the larger part was usually the basal cell. The tip cell was the one to divide first; the two resulting cells each divided again or only one divided. In one case the young sporophyte consisted of 5 cells, in the other of 4 cells; both conditions were found. The basal cell continued to grow. Most of its chloroplasts were grouped in the upper part of the cell. It then divided, leaving the lower cell without many chloroplasts. This cell then began to push downward at one side, becoming the first rhizoidal cell. It was not until the sporophyte was 6- to 12-celled that the cells began to divide in another direction. Those in the center of the plant were the first to divide vertically, thus causing the widening of the sporophyte just above the middle.

With Egregia, Myers reported the first vertical division taking place in the apical cell. In Nereocystis the apical cell did not divide until several of the central cells had divided.

The small plant was monostromatic until it had 40-50 cells; then the cells at the base began to divide in a third plane. Each of the basal cells sent down a rhizoid until there was a tangled mass. Where these rhizoids came in contact with the walls or sides of the dish they flattened slightly at the ends; sometimes they forked there. The monostromatic character of the few cells at the tip of the plant seemed to remain for some time; this part then died. The basal portion, which was then composed of several thicknesses of cells, formed a new tip not so acute as the old one.

When the small sporophyte was .5 mm long, the large cells of the inner web which gave rise to the sieve tubes were already formed. Some of them measured 141 by 45 micra; others were 45 micra square; and the surface cells in the rapidly dividing cortical areas were but 24.9 by 13 micra, to about 13 micra square. Sporophytes 1.5 cm long were found in cultures that were a year old. At this time the basal portion had become a decidedly conical disk; the stipe was apparent and the edges of the disk showed slight projections. The earliest signs of these projections were the pushing out of cells in certain regions beyond the border of the disk. These cells were quite evident in the early formation of the disk. The resulting crenate appearance in the slightly older disks seemed to be the result of apical growth from these cells. By the time the disk and its protuberances were well formed, the abundant rhizoids which had grown out from many of the surface cells and formed an intertwined mass, had disappeared. The rhizoids, as reported by Myers for Egregia, were without transverse walls and were unbranched, except for the above mentioned somewhat forked condition when they came in contact with the slides or sides of the dishes. Angst, however, reported cross walls in the rhizoids of Costaria. The small sporophytes grown in cultures as well as those collected in nature showed, upon microscopic examination of the surface, decided lines thru the frond emerging from the top of the stipe and radiating out fan-shaped thru the frond. These lines had the appearance of long inner web cells reaching the surface along certain lines and not covered with the smaller cortical cells.

In a cross section thru the small disk holdfast and thru one of the protuberances, the disk was seen as a solid growth of cells, of which only the outer ones had chromatophores. The inner colorless

ones from the crenate portion of the disk were elongated toward the center of the disk. A cross section above the basal portion showed the colorless cells to be iso-diametric. The hapteres are a later development, and to date have not appeared. It is probable, however, that these are formed in much the same way as the crenate margins of the disk.

On the whole Nereocystis differs from the Laminariaceae that have been previously described in that it has a gametophyte growth of much longer duration before maturation, and that its antheridial gametophyte is much more branched and extensive than those described for the other forms. It has an indefinite reproductive period. That the gametophyte can live thru several years has not been tested for others of the group.

Summary

Nereocystis has differentiated male and female gametophytes with much branched and extensive filaments. The gametophyte plant has the power of vegetative reproduction and of continuing its reproductive processes for at least a year.

The gametophyte takes between 10 and 11 weeks to reach maturity.

Cultural sporophytes have been grown to 1.5 cm in length and have developed a primary disk, a holdfast, stipe, and frond which lost its monostromatic character.

Summary of Process of Growth

24 hours; spores started germinating.

48 hours; cell contents passed into the new ends of the filament.

1 week; gametophytes of 2 cells.

2 weeks; gametophytes of 3 cells.

3 weeks; gametophytes of 4-8 cells; one or two individuals started to extend cells laterally as in branching.

4 weeks; plants showed many cells and more branching.

5 weeks; many branches present; basal cells of the gametophytes began to divide and branch.

6 weeks; filaments were still lengthening and branching profusely.

7-8 weeks; filaments were becoming complex, almost plate-like in some cases; differentiation of gametophytes became apparent.

9 weeks; sexual differentiation was more decided.

10-11 weeks; oogonia and antheridia present; sporophytes began to appear.

12 months; sporophytes were still forming; all stages of growth were found in the cultures.

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NEREOCYSTIS LUETKEANA

Fig. 1. Zoospores. ×650.

Fig. 2. Germinating spore. ×650.

Fig. 3-7. Stages in the germination of the spore, ×650; x, spore end.

Figs. 8-13. Progressive steps in the growth of the one celled gametophyte. ×650.

Figs. 14-16, 23. Two celled gametophytes. ×650.

Figs. 17-23. A group of gametophytes from a three weeks culture showing various stages of germination in the same culture. ×650.

Figs. 24-26. Four celled gametophytes. ×650.

Figs. 27-30. Later stages in the undifferentiated gametophyte. ×650.

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Figs. 31, 34. Male gametophyte when differentiation becomes apparent. ×650.

Figs. 32, 35, 36, 37. Male gametophytes in the process of forming antheridia at the tip of the branches. ×800.

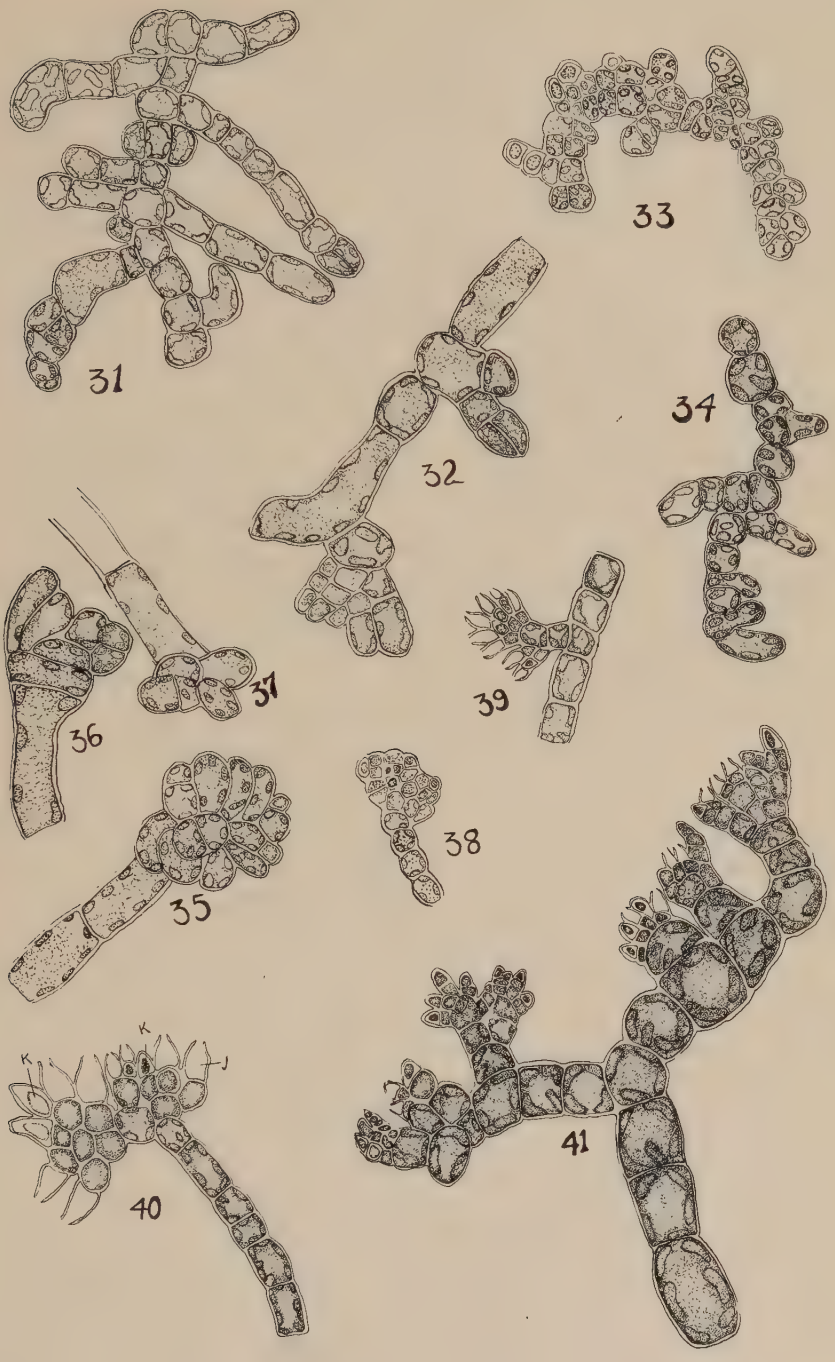
Fig. 33. Male gametophyte in older cultures, almost thalloid in appearance, each cell becoming an antheridium. ×650.

Fig. 38. Antheridia forming at tip of a male gametophyte thread. ×650.

Fig. 39. Antheridia forming on side branches of the male gametophyte filament. ×650.

Fig. 40. Group of antheridia at tip of an antheridial filament showing (j) empty cells where sperm has escaped (k) sperms in antheridia. ×650.

Fig. 41. More complex antheridial filament. ×800.



NEREOCYSTIS LUETKEANA

Nereocystis luetkeana

Fig. 42. Female gametophyte. ×250.

a - Empty oogonium. d - Oospore.

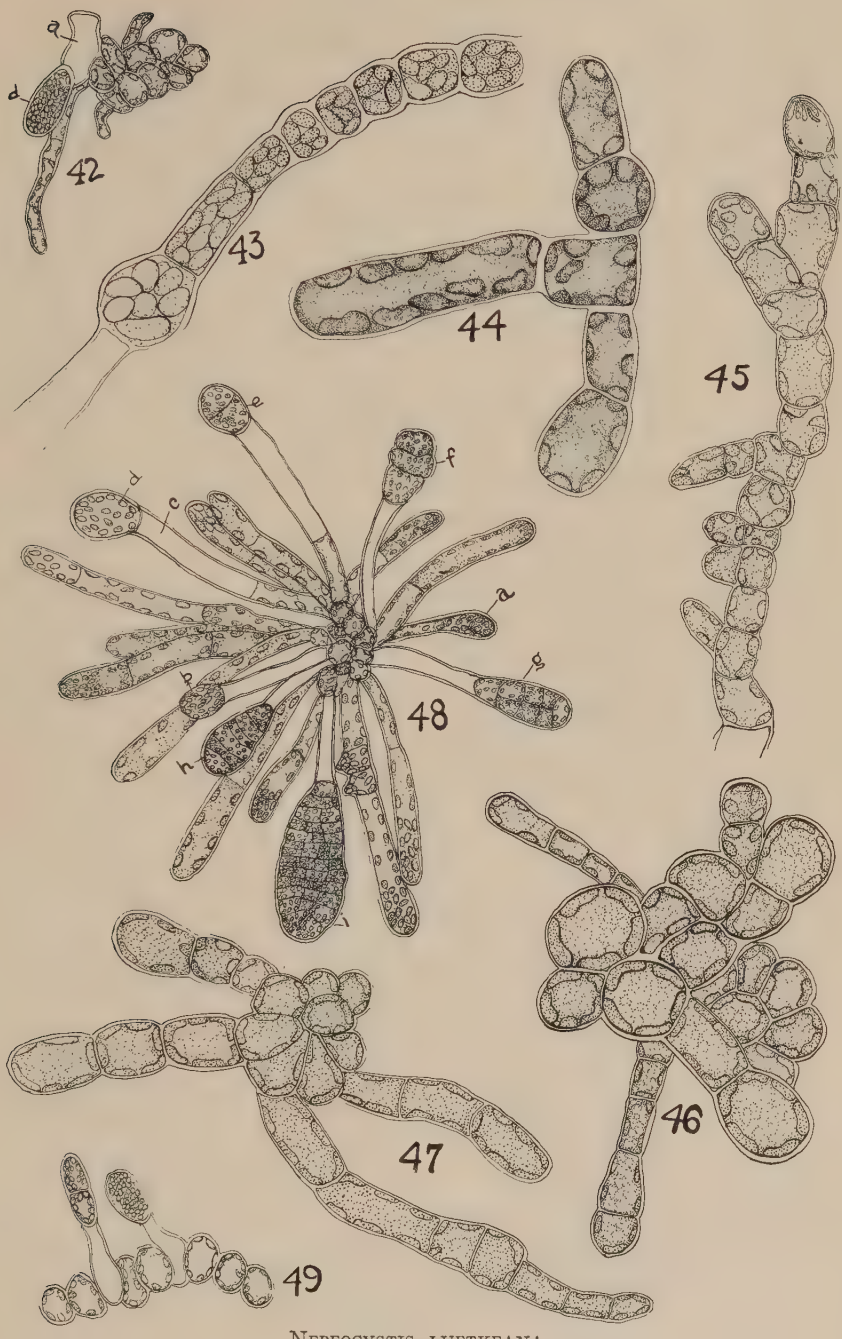
Fig. 43. Female gametophyte in older cultures when nearly every cell is spherical. ×650.

Figs. 44-47. Female gametophyte at time of differentiation. ×650.

Fig. 48. Female gametophyte showing the rosetted appearance. ×250.

a - Oogonium
b - Egg
c - Empty oogonium
d - Oospore
e - Two celled sporophyte
f - Three celled sporophyte
g, h, i - Various stages in the sporophyte growth

Fig. 49. Female gametophyte of older cultures. Every cell may produce a sporophyte. ×250.



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NEREOCYSTIS LUETKEANA

Fig. 50. Portion of a female gametophyte sporophyte. ×250.

a - Gametophyte
b - Empty oogonium
c - Sporophyte

Figs. 51, 54, 55-57, 59. Female gametophytes with adherent sporophytes in various stages of development. ×250.

Fig. 52. Sporophyte. ×650.

a - Rhizoids

Fig. 53. Female gametophyte with sporophyte. ×650.

Fig. 58. Group of sporophytes. ×250.

NEREOCYSTIS LUETKEANA

Nereocystis luetkeana

Fig. 60. Beginning of primary disk holdfast. × 250.

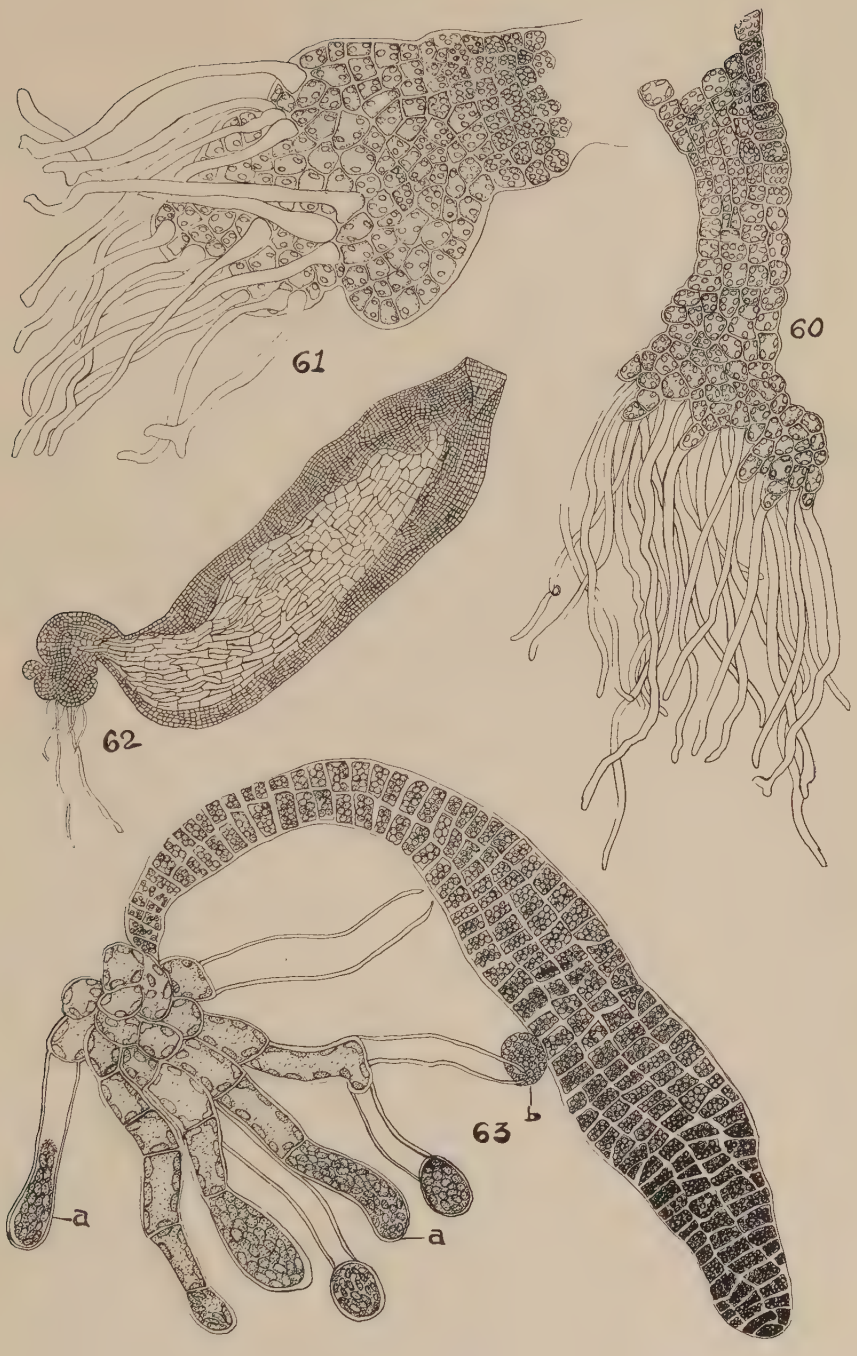
Fig. 61. Later stage in the formation of disk. ×250.

Fig. 62. Plant grown in cultures (1 cm long) showing large inner web of cells and outer cortical layer of much smaller nearly isodiametric cells. ×90. (Cells, in part diagrammatical).

Fig. 63. Female gametophyte with sporophyte. ×650.

a - Oogonium before discharging the egg

b - Egg just after emptying from oogonium



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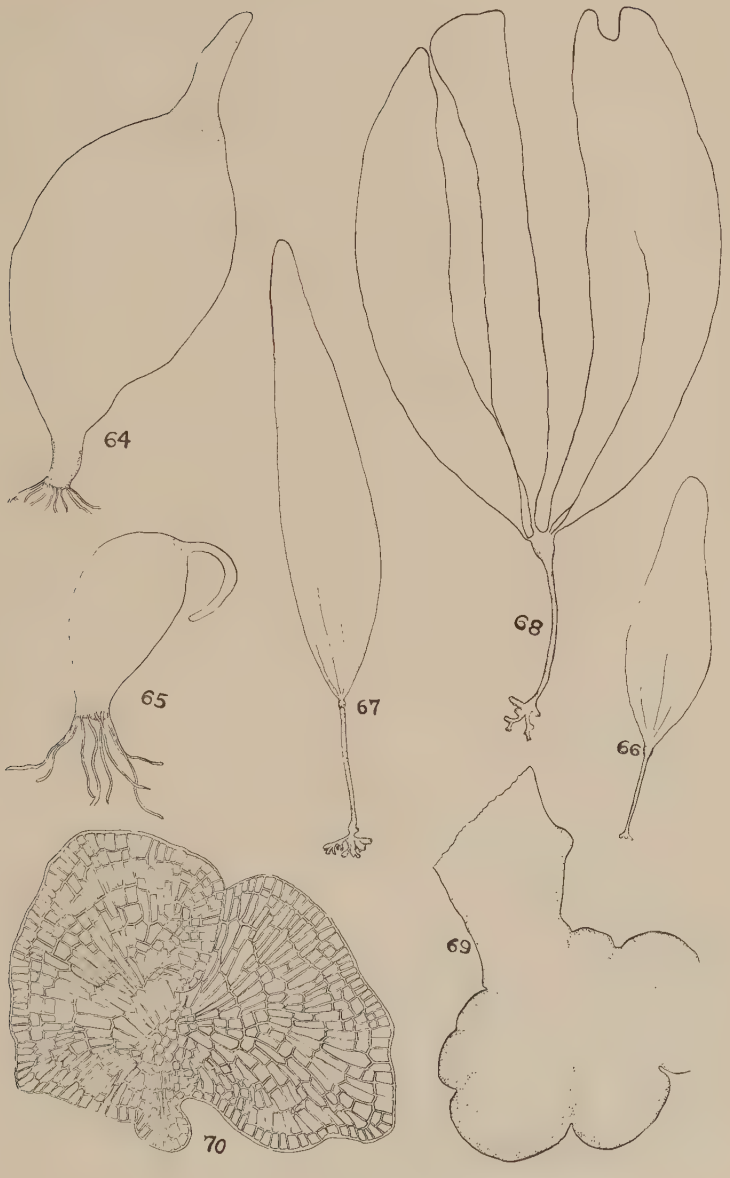
NEREOCYSTIS LUETKEANA

Figs. 64-65. Outline of sporophytes. The narrower portion at tip is monostromatic. ×215.

Figs. 66, 67, 68. Forms found in nature showing development of holdfast, pneumatocyst, and splitting lines of fronds. ⅓ nat. size.

Fig. 69. Primary disk on plant in year old cultures. Plant measures 1.5 cm in length. ×77.

Fig. 70. Freehand section through above disk. ×77.



NEREOCYSTIS LUETKEANA

Nereocystis luetkeana

Fig. 71. Microphotograph of Nereocystis taken from a culture five months old. ×500.

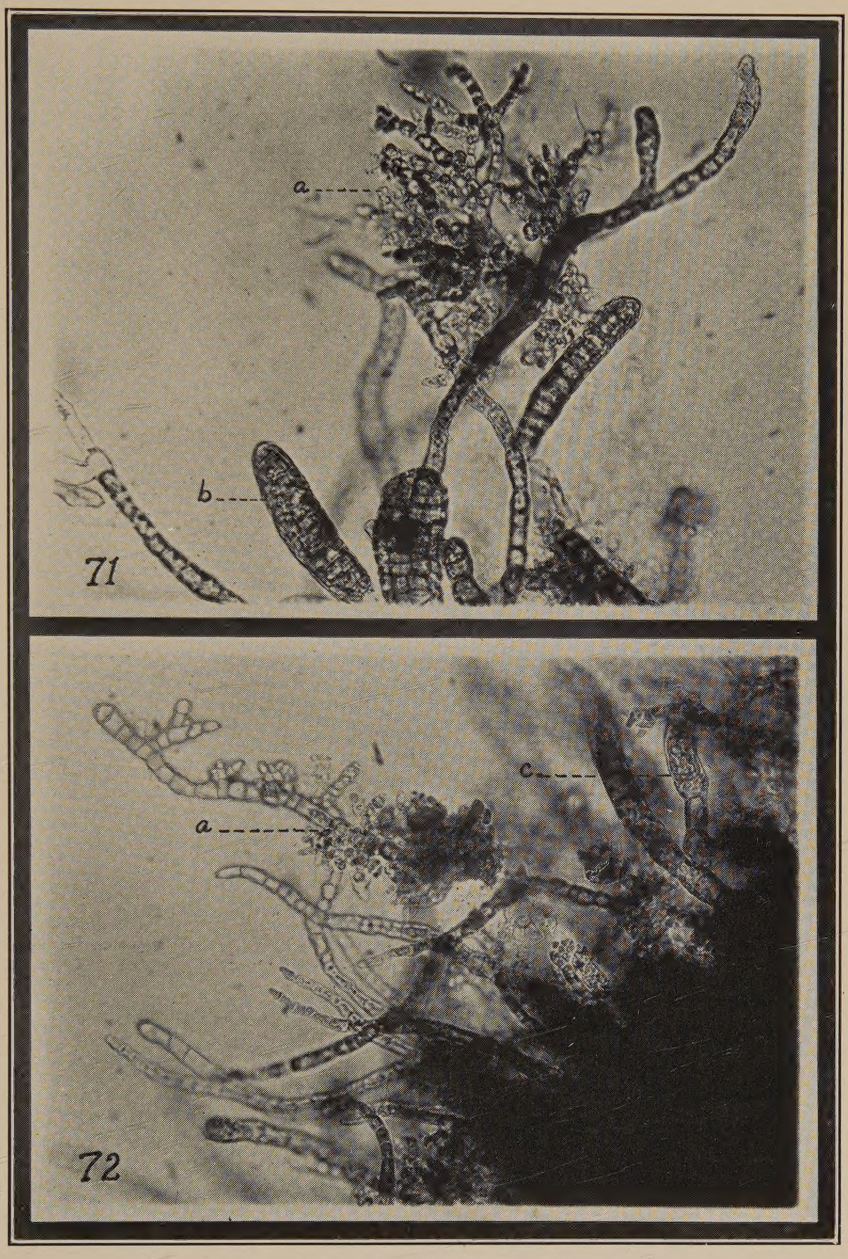
a - Male gametophyte with antheridial cluster

b - Sporophyte.

Fig. 72. Microphotograph of Nereocystis. ×500.

a - Antheridia shown coming from side branches in small clusters

c - Oogonium



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